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A New Trigger for T Cells

Minireview

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Understanding the early events in T cell activation and signaling is an active area of research. A recent study (Gil et al., 2002) has described a new trigger for T cell activation, involving a TCR-ligand-induced conformational change in CD3ϵ that permits binding of the adaptor protein Nck.

T cell recognition is a central part of the immune response to foreign entities. Mediating this recognition are T cell receptor (TCR) heterodimers, which are like antibodies in their ability to create vast numbers of different variable (V) region sequences through DNA rearrangement and other mechanisms (Davis et al., 1998). Unlike antibodies, T cell receptors always remain on the cell surface, and the major species, αβ type TCRs, recognize specific fragments of antigens (peptides) bound to major histocompatibility complex (MHC) molecules and displayed on the surfaces of other cells. This ability to survey degradation products of antigens makes for a detection system that is intrinsically difficult for pathogens to avoid, as all proteins must eventually be degraded. It is also an extremely sensitive system, as very few peptide-MHC complexes on an antigen-presenting cell are needed to trigger a T cell response. In order to understand how engagement of the TCR to a ligand activates a T cell, attention has long focused on the CD3 molecules (CD3γ, δ, ϵ, and ζ) which are assembled together with the TCR heterodimer and possess a characteristic sequence motif for tyrosine phosphorylation, known as ITAMs (Reth, 1989). The TCR polypeptides themselves have very short cytoplasmic tails, and it appears that all proximal signaling events are mediated through the CD3 molecules. As shown in Figure 1A, they are organized in dimeric units (CD3γϵ, CD3δϵ, and CD3ζζ) (Terhorst et al., 1996), and evidence also indicates that there are two TCR heterodimers per unit (Fernandez-Miguel et al., 1999, Terhorst et al., 1996). The variety and apparent redundancy of these polypeptides probably relate to the varying signals that a T cell must receive in order to mature and survive. In particular, immature T cells are heavily selected in the thymus both for a successful TCR β rearrangement (the pre-TCR-CD3 complex) and later for complete αβ TCRs which react weakly to self-peptide-MHC complexes, which is important for avoiding selection of self-reactive T cells. The selected T cells then require additional stimulation in the periphery from such ligands in order to survive. This selection and maintenance process is apparently necessary to establish a TCR repertoire that is both predisposed toward a particular MHC complement and in a high state of readiness to respond to the appropriate foreign antigen when it comes along.

For many years it has been thought that the principal trigger for T cell activation is the tyrosine phosphorylation of CD3 molecules, particularly CD3ζ, brought about by TCR crosslinking and mediated by lck (Weiss 1999). The recent report of Alarcon and colleagues (Gil et al., 2002) adds a new and unexpected element to the story, with a very strong case for an additional and slightly earlier triggering event involving a conformational change and subsequent exposure of a proline-rich region of the cytoplasmic tail of CD3ϵ. Exposure of this region allows the adaptor protein Nck to bind. They demonstrate that this can occur in isolated TCR-CD3 complexes, even, surprisingly, with stimulation by monvalent antibodies, and that it seems completely independent of early tyrosine phosphorylation. The association of Nck to CD3ϵ appears to be necessary for proper T cell activation, as a dominant negative construct of Nck inhibits the ability of T cells to form conjugates to other cells in response to antigen, and disrupts aspects of synapse formation, cytoskeletal reorganization, and cytokine production. Thus, it appears that there are at least two distinct pathways whereby TCR engagement triggers activation in T cells: tyrosine phosphorylation of ITAMs by Lck and Fyn, and a conformational change in CD3ϵ leading to Nck recruitment, with both of these occurring within seconds of TCR engagement with ligand. These signals probably converge at some point downstream, with a likely point of convergence being the signaling molecule SLP-76. SLP-76 is an adaptor protein which is essential for T cell activation, reportedly binding to Nck, ZAP-70, and Vav (a guanine nucleotide exchange factor) (Lin and Weiss, 2001).

Possible Mechanisms

What exactly happens to CD3ϵ to expose the proline rich region to Nck binding is not clear. Structural information on the CD3 molecules is very sparse (Sun et al., 2001) and only pertains to the external domains. Interestingly, Stern and colleagues (Aivazian and Stern, 2000) found that in a lipid environment, the cytoplasmic region of CD3ζ is helical, but that it becomes disordered when phosphorylated. They have proposed that this is a trigger for activation, but there is as yet no evidence of this happening within a cell. Gil et al. propose that TCR engagement induces a conformational change in CD3ϵ that allows Nck binding and show that the binding of some antibodies, but not others, to TCR-CD3 exposes the Nck binding site on isolated complexes. One caution that should be stated here about these experiments is that exposure to the natural ligand (peptide-MHC) is not used here, but rather, anti-CD3 antibodies and Fab′ fragments of the antibodies are used as the stimulus. At least one of these antibodies is directed against CD3ϵ directly, and this may enhance the effect seen. Still, in other experiments, Gil et al. show that the exposure of TCR transgenic mouse T cells to antigen-pulsed presenting cells promotes Nck binding to the TCR/CD3 complex, which helps to allay concerns about the artificiality of the antibody induction. It should also be noted

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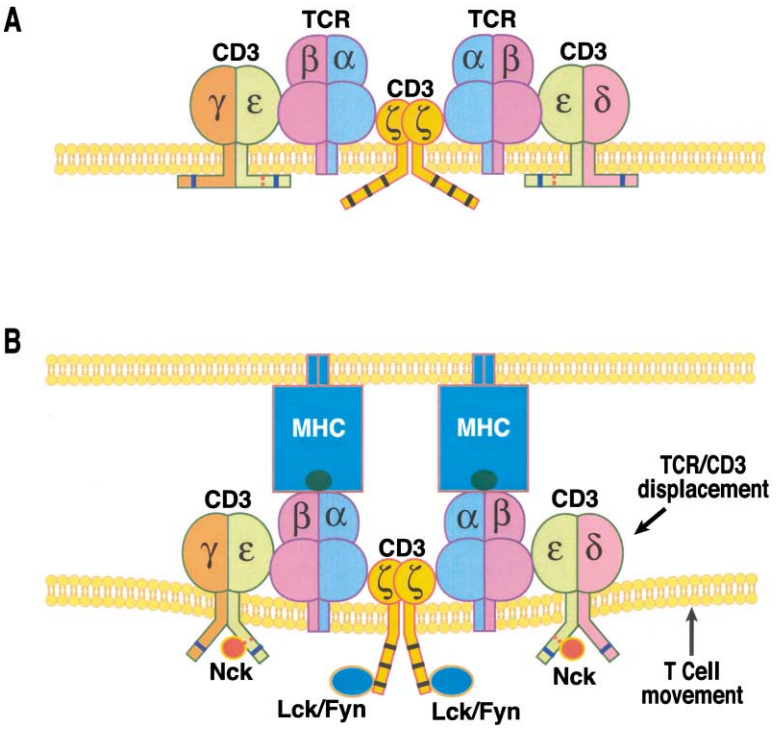

Figure 1. Models for the Organization of TCR/CD3

(A) Approximate organization of TCR/CD3 subunits from Terhorst et al. (1996) and Fernandez-Miguel et al. (1999), incorporating the concept that the cytoplasmic domains of some or all of the CD3 chains are stabilized into a helical conformation by their proximity to the plasma membrane (Aivazian and Stern, 2000; Gil et al., 2002).

(B) Postulated ligand engagement may change the relationship of both CD3ζ and ϵ cytoplasmic regions, exposing them to the src kinases Lck and Fyn and Nck interaction, respectively. This may occur by either a piston-like conformational movement or a mechanical displacement of the entire complex inward because of T cell pressure on the antigen-presenting cell, or both.

that attempts to trigger even the most sensitive helper T cells with monovalent ligands have been unsuccessful (Cochran et al., 2000; Boniface et al., 1998), with only dimers or trimers of peptide-MHC being effective. Thus, while the triggering pathway described by Gil et al. may be necessary for complete T cell activation, it doesn't appear to be sufficient. As the authors discuss, this finding is in apparent contradiction to structural data showing that no conformational changes in the membrane proximal portion of the TCR occur with binding (as reviewed by Rudolph and Wilson, 2002), although changes in the binding site can be considerable (Reiser et al., 2002, Wu et al., 2002). Still, there are precedents in other receptor systems where ligand engagement changes the relationship between components. As discussed by Gil et al., following earlier suggestions by Sun and colleagues (Sun et al., 2001), one attractive possibility comes from the work of Otteman et al. (1999), who showed by spin labeling that the rod-like extracellular components of the aspartate receptor move in a piston-like fashion upon ligand binding. While the displacement was small (approximately 1 Å), it might be larger in the case of TCR/CD3 complexes and change the relationship of the cytoplasmic domains of CD3ϵ with respect to the membrane. Although not very similar in terms of sequence with CD3ζ, if the effect seen by Aivazian and Stern (2000) for CD3ζ has relevance for CD3ϵ, then its displacement with respect to the membrane might radically change its structure and promote Nck binding. It is also possible that the relatively low affinity (micromolar) of TCR ligand interaction is not sufficient to induce CD3ϵ exposure, and it also requires some mechanical force on the part of the T cell, pressing against the surface of the antigen-presenting cell. This could augment a "piston" mechanism, in which all or part of the TCR-CD3 complex is displaced into the membrane. This possibility is summarized in Figure 1B, which draws on the findings of Gil et al., as well as the prior speculations of Otteman et al. (1999), Aivazian and Stern (2000), and Sun et al. (2001). Alternatively, ligand binding has been seen to induce very large allosteric changes in a number of receptor complexes, including the anaturetic peptide receptor (He et al., 2001) and a bacterial ribose binding protein (Mowbray and Bjorkman 1999). So, perhaps changes of this sort can be induced in CD3 molecules by some movement or rotation of the TCR upon ligand binding that is not readily apparent from the liganded and unliganded structures of the extracellular portion, but that somehow changes its relationship to the CD3 molecules. Another possibility is that slowing the lateral mobility of the TCR through binding to its ligand embedded in another membrane might change its lipid environment and that of the CD3 molecules associated with it (van der Merwe, 2001).

In summary, Gil and colleagues describe a completely new pathway in T cell activation, and trace it directly to a little-understood member of the TCR/CD3 complex, CD3ϵ. This pathway seems entirely independent of the aggregation-dependant tyrosine-phosphorylation of CD3 molecules, at least in its early stages, which up until now has been thought to be the principle trigger of cellular activation. Hopefully we will learn, in the not-so-distant future, the precise structural and dynamic relationships between the molecules of this large receptor/signaling complex, and how the different components change upon ligand binding, as well as the subtle

(or perhaps not so subtle) variations that may occur during T cell development and in peripheral maintenance.

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